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An Intracerebral Probe with Integrated 10×1 µLED Array for Optogenetic Experiments at 460 nm

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Abstract

Optical stimulation and inhibition of neural tissue is a promising neuroscientific method to control neural activity in so-called optogenetic experiments. This work illustrates the development of an implantable neural probe with an array of 10 integrated light emitting diode (µLED) chips emitting at a wavelength of 460 nm. The probe is suitable for experiments requiring a depth-controlled optical stimulation at multiple sites. The µLEDs are flip-chip-bonded to a polyimide cable stiffened by a micro-machined ladder-shaped silicon element for robust insertion into neural tissue. The optical operation of the 250-µm-wide, 65-µm-thick and up to 8-mm-long devices and their stability in an electrophysiologically relevant electrolyte is demonstrated. This tool paves the way for a large variety of optogenetic experiments in neuroscience.

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Keywords: Optogenetics; 460nm; µLEDs; Neural probe; Integrated light source

1. Introduction

Optogenetics is a relatively new experimental approach that enables neuroscientists to optically interact with neural tissue using light sensitive proteins, i.e. so-called opsins, integrated into the membrane of neurons by viral transfection. As an example, channelrhodopsin-2 (ChR2) serves as a light gated ion channel which is most sensitive for blue light around 470 nm [1]. Typically, light from an external light source, i.e. laser or high-power LED, is delivered to the neural tissue using optical fibers [2], while the neural activity is monitored by silicon-based probe arrays {Fig. 1(a)}. However, the mechanical stiffness of these fibers potentially influences freely behaving animals. In order to

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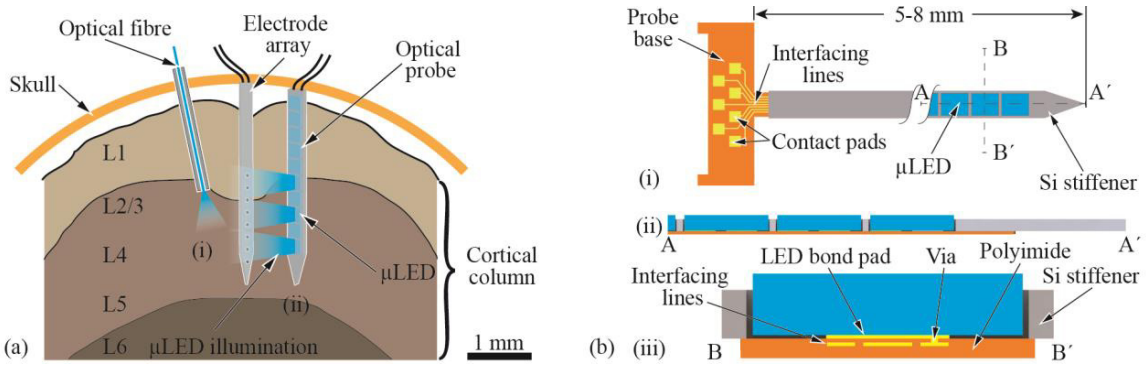

Fig. 1. (a) Schematic of the experimental situation illustrating a neural probe, i.e. electrode array, adjacent to (i) an optical fiber and (ii) a μLED-based probe providing a depth controlled illumination of brain tissue; (b) schematic of μLED-based optical probe in (i) top view and (ii,iii) cross-sectional views along lines (ii) A-A' and (iii) B-B' as indicated in (i).

circumvent this drawback, intracerebral probes carrying electrode arrays can be equipped with integrated light sources and waveguides [3]. Further, a depth-controlled optical stimulation of neural tissue is requested in order to analyze translaminar interactions in the cortex. This can be achieved by multiple optical fibers or a fiber translated inside the neural tissue using a micro-drive [4]. However both options are expected to result in unacceptable tissue damage. As illustrated in Fig 1(a), a linear array of μLEDs integrated along a slender probe substrate will enable the laminar stimulation of the cortex. In contrast to Ref. 5 requesting an improved mechanical flexibility for probe implantation into the cochlea, the optical probe for cortical applications has to be mechanically stiffened to enable probe insertion into deeper brain regions.

2. Probe design

Figure 1(b) illustrates the components of the laminar optical probe developed in this work. The probe comprises a substrate with a thickness between 7.5 μm and 11.5 μm based on polyimide (PI), and metal structures serving as leads, LED bond pads and contact pads for the electrical interconnection to the external instrumentation. Commercial μLED chips (TR2227 CREE Durham, NC; centre wavelength 460 nm, size 270×220×50 μm^3) are integrated along the PI substrate at center-to-center distances of 300 μm and 500 μm. The probe is stiffened using a micro machined ladder-shaped silicon (Si) structure with openings gathering the μLEDs. As illustrated in the cross-sections in Figs. 1b (ii) and (iii), the resulting optical probe assembly measures 65 μm in thickness and 240 μm in width, which makes it suitable for implantation into brain tissue.

The probes have been designed in two different shank lengths of 5 mm and 8 mm. Furthermore, considering the delicate assembly process, the stiffener design has also taken into account the μLED size tolerances and the ladder wall widths.

3. Fabrication and Assembly

3.1. Processing of PI substrate and stiffener

The process sequence of the PI substrate is summarized in Fig. 2. It applies a first 5-μm-thick PI layer (U-Varnish S, UBE Industries Ltd., Tokyo, Japan) spin-coated onto 4-inch Si wafers {Fig. 2(a-i)}. This is followed by depositing a seed layer {30 nm chromium (Cr), 200 nm gold (Au)} and electroplating the probe metallization (1 μm Au) required to lower the resistance of the 10-μm-wide interconnecting lines {Fig. 2(a-ii)}. Subsequently, the seed layer is removed in a maskless process using wet etching followed by the deposition of a second, 2.5-μm-thick PI layer. Thereafter vias are etched onto the first metal layer by reactive ion etching (RIE), and a second metallization used again as a seed layer is patterned and electroplated locally to a thickness of 3 μm {Fig. 2(a-iii to v)}. Finally, the probe shape is patterned by RIE using photoresist as masking layer before the probes are peeled off using tweezers {Fig. 2(a-vi to viii)}.

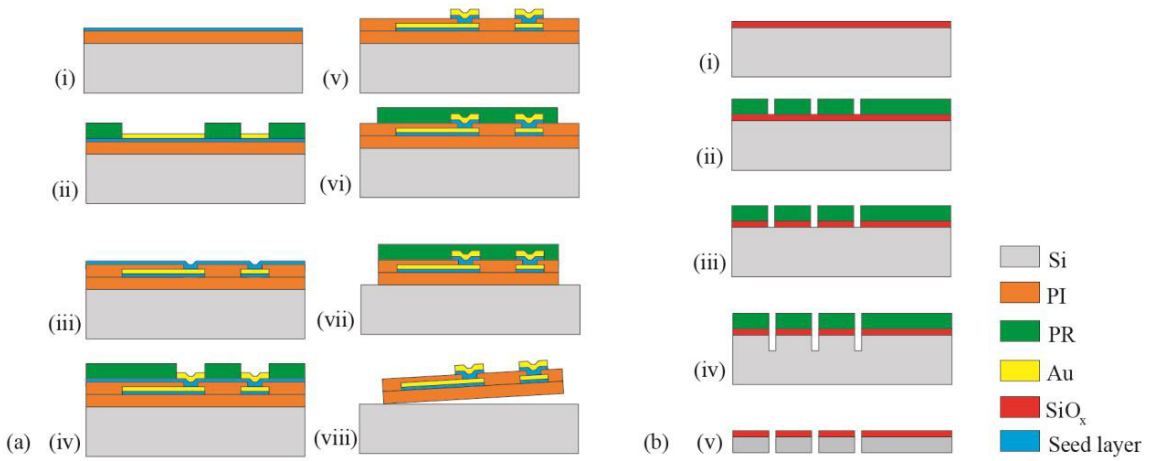

Fig. 2. (a) Fabrication process of PI cables using (i, iii) spin-coating of PI and seed layer sputter deposition, (ii, iv) electroplating, (ii, iv, vi) photolithography, (vii) PI patterning by reactive ion etching, and (viii) probe peel-off. (b) Fabrication sequence of Si stiffener based on (i) SiO_x masking layer deposited by PECVD, (ii) photolithography, (iii) RIE of SiO_x, (iv) DRIE of Si, and (v) wafer grinding.

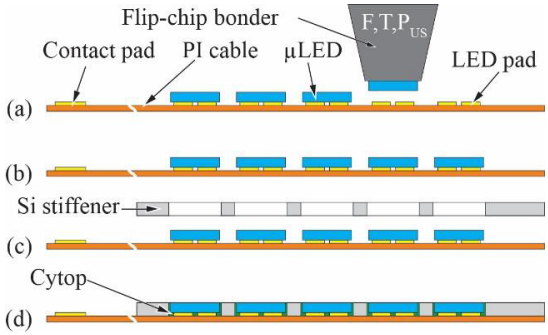

Fig. 3. System assembly based on (a) flip-chip bonding of LED chips and (d) adhesive fixation of Si stiffener.

As illustrated in Fig. 2(b), the Si-based stiffener is realized on 4-inch Si comprising a 1-µm-thick silicon oxide (SiO_x) deposited using plasma enhanced chemical vapor deposition (PECVD). This oxide layer is patterned by RIE and acts as an etch mask for the subsequent deep reactive ion etching (DRIE) of silicon {Fig. 2(b-iii and iv)}. Following the DRIE process with an etch depth of 60 µm, the wafers are ground to the requested stiffener thickness of 40 µm. Overall this process is similar to the etching-before-grinding (EBG) process [6].

3.2. Probe Assembly

The probe assembly is a delicate process with regards to alignment accuracy. Its multiple steps comprise µLED flip-chip bonding, stiffener assembly, polymeric under-fill and encapsultion. For µLED bonding, the peeled-off PI substrates are fixed on a custom-made Si vacuum chuck. The µLED chips are then individually aligned over the substrate and flip-chip bonded to the bonding pads using ultrasonic power, temperature and pressure, as shown in Fig. 3(a) and (b). The silicon stiffener is then carefully positioned over the bonded µLED probe with the help of tweezers {Fig. 3(c)}. The µLEDs are then underfilled by dispensing a small amount of the fluoropolymer CYTOP CTL-809M (Asahi Glass Co. Ltd., Japan) which acts as well as an adhesive between stiffener and PI substrate. The assembly depicted in Fig. 3(d) is then subjected to a temperature profile reaching 120 °C for CYTOP curing. In view of neuroscientific experiments the probes need to be further protected against the electrophysiological environment by a transparent coating. This is achieved by exposing the probes to an oxygen plasma for surface activation and subsequently dip-coating them into CYTOP for passivation, which is followed by another curing step.

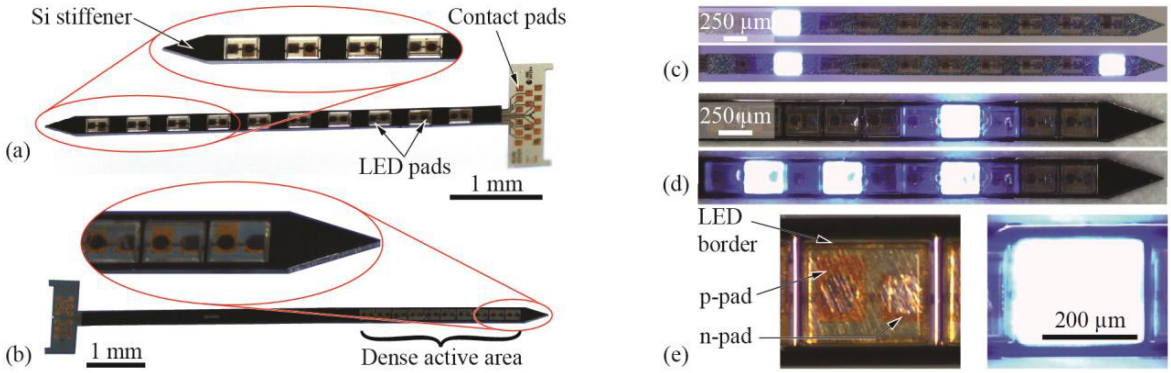

Fig. 4: Micrographs of actual devices showing (a) 5-mm-long probe with sparsely integrated LEDs, (b) 8-mm-long probe with a dense arrangement of LEDs, (c,d) single and multiple LEDs in the on-state, and (e) detailed view of an LED switched off and on.

4. Characterization

Figures 4(a) and (b) show 5-mm-long and 8-mm-long versions of the optical probe with sparse and dense arrangements of µLEDs, respectively. Figure 4(c) demonstrates the selective illumination of single and multiple µLEDs while Figs. 4(d) and (e) provide more detailed views of µLEDs switched off and on.

In order to evaluate the probe stability under electrophysiological conditions, assembled probes were tested in Ringer's solution over a period of 40 hours. A reverse voltage of 5 V was applied across the system, which corresponds to approximately twice the forward voltage requested for light emission of a µLED. With the µLED immersed in Ringer's solution, the current was recorded vs. time. In this worst case testing, the probes showed an acceptable stability for acute animal experiments with a reverse current below 2.8 µA. Furthermore, the optical power output was measured under modulated operation at 10 kHz and 5% duty cycle to be 107.2 µW at a forward current of 4.8 mA.

Conclusions

In this paper, we presented an intracerebral probe dedicated for a depth-controlled optical stimulation of the cortex suitable for optogenetic experiments. The probes incorporate integrated µLEDs at two spatial resolutions with a high optical power output. The electrical insulation of the probe is proven suitable for acute animal experiments. The dispersion of light in animal brain tissue is a further subject of interest. In vivo recordings are underway.

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References

[1] E. S. Boyden, F. Zhang, E. Bamberg, G. Nagel and K. Deisseroth, Millisecond-timescale, genetically targeted optical control of neural activity, Nature Neuroscience, Vol. 8, 2005, pp. 1263-1268.
[2] O. Yizhar, L. E. Fenno, T. J. Davidson, M. Morgi and K. Deisseroth, Optogenetics in neural systems, Neuron, Vol. 71, 2011, pp. 9-34.
[3] M. Schwaerzle, K. Seidl, U. T. Schwarz, O. Paul and P. Ruther, Ultracompact optrode with integrated laser diode chips and SU-8 waveguides for optogenetic applications, Proc. IEEE MEMS Conf. 2013, pp. 1029-1032.
[4] P. Anikeeva, AS. Andalman, I. Witten, M. Warden, I. Goshen, L. Grosenick, LA. Gunaydin, LM. Frank, K. Deisseroth, Optetrode: A multichannel readout for optogenetic control in freely moving mice. Nat Neurosci, Vol 15: pp. 163-170
[5] C. Goßler, C. Bierbrauer, R. Moser, M. Kunzer, K. Holc, W. Pletschen, K. Köhler, J. Wagner, M. Schwaerzle, P. Ruther, GaN-based micro-LED arrays on flexible substrates for optical cochlear implants. J. Physics D: Appl. Physics, Vol. 47, 2014, 205401 (6pp)
[6] S. Herwik, O. Paul and P. Ruther, Ultrathin silicon chips of arbitrary shape by etching before grinding. J. Microelectromech. Syst., Vol. 20, pp. 791-793